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Tailflick Escape Behavior in Larval and Juvenile Lobsters (*Homarus americanus*) and Crayfish (*Cherax destructor*)

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Abstract. We examined the escape behavior of larvae and postlarvae of the American lobster (*Homarus americanus*) and of adult immature (stage ADI) crayfish (*Cherax destructor*). Responses to standardized water jet stimuli delivered through a pipette were observed and analyzed. Lobster larvae did not respond to stimuli within 60 ms, indicating that they do not have functional giant fibers. The first movement by lobster larvae in response to water jet stimuli was a hyperextension of the abdomen. Larval escape responses also showed very little habituation. Postlarval lobsters and ADI crayfish showed the same range of responses as adult animals. Displacement efficiency of tailflicks exhibited by the different animals and stages was examined and related to the morphology of the animals. A separate behavior from tailflicking by larval lobsters in response to water jet stimuli was also observed. Here, the abdomen was hyperextended and the thoracic appendages were promoted. We termed this behavior a "starburst" response. The features of the tailflicking behavior suggest that it evolved to make the larvae difficult prey to handle for small, slower moving predators, and possibly to allow them to ride the bow waves of faster moving predators.

Introduction

Adult lobsters (*Homarus americanus*) and crayfish (*Cherax destructor*) escape from threats by predators and conspecifics by executing rapid, rhythmic flexions and extensions of the abdomen in a behavior commonly called tailflicking. This behavior has been studied over several decades both because of its intrinsic importance to the behavioral ecology of the animal and because of the insights it can provide into the neuroethology of escape (see recent review by Edwards *et al.*, 1999). Crayfish, mainly *Procambarus clarkii,* have been particularly well studied (last reviewed by Wine and Krasne, 1982), but there are also data on some other species such as the Norway lobster (*Nephrops norvegicus:* Newland and Neil, 1990), the hermit crab (*Pagurus pollicarus:* Umbach and Lang, 1981), the American lobster (*H. americanus:* Davis and Davis, 1973; Govind and Lang, 1976; Lang *et al.*, 1977) and the Australian crayfish (*C. destructor:* Cooke, 1985; Cooke and Macmillan, 1985; Davey and Macmillan, 1991).

Because of this extended series of investigations, we know a great deal about tailflicking behavior and its neuronal basis. Three types of escape tailflick have been described: the medial giant (MG) tailflick, the lateral giant (LG) tailflick, and the nongiant (NonG) tailflick. The names refer to the involvement or otherwise of the giant axon, or fiber, systems that run the length of the ventral nerve cord in many malacostracan crustaceans. The medial giant fibers (MGFs) are activated by threatening stimuli to the head and anterior end of the animal. The action potentials generated travel posteriorly, synapsing as they go with sets of neurons that promote the legs and flex all the abdominal segments. The consequence is an MG tailflick in which the body is streamlined and propelled linearly backwards. The lateral giant fibers (LGFs) are excited only by mechanical stimuli to the tail and posterior end of the animal. Action potentials in the LGFs travel anteriorly but do not cause contractions in the last two abdominal segments (*i.e.,* the first two segments through which they pass). Because only the ante-

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Abbreviations: ADI, adult immature; LG, lateral giant; LGF, lateral giant fibers; MG, medial giant; MGF, medial giant fibers; NonG, nongiant.

rior abdominal segments contract during an LG tailflick, the animal pitches forwards and upwards so that its trajectory carries it appropriately away from the vicinity of the threat (Mittenthal and Wine, 1973). The giant fibers appear to fire only once during any given escape episode, producing either a single tailflick or the first in a series of tailflicks in which subsequent tailflicks do not involve the giant fibers (Shrameck, 1970; Wine and Krasne, 1972; Cooke, 1985). Tailflicks occurring in the absence of giant fiber activity are called nongiant tailflicks. Because of the large diameter and concomitant fast conduction velocity of the giant axons, there is no time for sensory feedback during tailflicks they trigger. Giant mediated tailflicks are therefore stereotyped, open-loop behaviors. Nongiant tailflicks occur with a number of sensory feedback loops operating, so they can vary in form. They can, for example, somersault an animal back to an upright position following an LG tailflick, move an animal in a curved path away from a threat, or otherwise adjust the orientation of the animal in three-dimensional space. Tailflicking escape behavior habituates rapidly to repeated stimuli, so that adult animals typically fail to produce a response to the fifth stimulus in a sequence of stimuli spaced minutes apart (Krasne and Woodsmall, 1969).

Distinguishing between the three types of tailflick while observing freely behaving animals is not necessarily easy. It is not yet clear how often giant fiber tailflicks are produced during extended periods of intermittent tailflicking such as occur during antagonistic encounters between conspecific males. LG flips are sometimes distinguishable when the characteristic LG body position occurs in the first tailflick in a series of flips. MG and NonG tailflicks are not distinguishable on body position alone. If the exact time at which a tailflick was initiated by the threatening stimulus is known, as in a contrived experimental situation, it is possible to distinguish between giant and nongiant tailflicks on the basis of the latency to the onset of movement. If movement occurs within about 20 ms of the stimulus, the tailflick must necessarily have been generated by giant fiber activation. Nongiant tailflicks appear only after about 60 ms, and usually after considerably longer (150 ms) intervals (Wine and Krasne, 1972).

The general features of escape tailflicking discussed above come from studies of adult animals. But behaviors that have evolved to increase the fitness of adults may not be appropriate for juveniles or larvae, which may face different predators and operate in environments in which the physically and biologically significant features may be spatially and temporally different. Lang *et al.* (1977), for example, found that the threshold for triggering an escape response in juvenile American lobsters slowly increased as the animals grew and as they developed larger claws with which to defend themselves. Accompanying this change was an increasing tendency to move into a defense posture when threatened. Fricke (1984, 1986) described neurophysiological changes accompanying these behavioral ones. Morphological changes during development are a particularly important consideration in an animal like *H. americanus* in which the first three stages are larval and pelagic. Not only are the body proportions different than in later stages, but they swim in an entirely different manner, using the exopodites of the walking legs rather than the pleopods of the abdomen as small juveniles do (Herrick, 1909). These exopodites are lost on the molt from the larval stage III to the first postlarval stage IV. In contrast, *C. destructor* undergoes direct development. It has no larval stages, and the young have the same body shape as the adult, albeit with different body proportions. In both species, the larvae and juveniles are small, have different body proportions and locomotory patterns and potentially different predators, so one would expect the evolution of different escape behavior patterns from those exhibited by the adult.

There is an extensive literature on the large-scale movements of planktonic animals associated with their vertical migration, aggregation, dispersal, and settlement, but recent studies have highlighted the importance of small-scale behavioral responses to their fitness and survival (Keough and Downes, 1982; Haury and Yamazaki, 1995; Lenz *et al.*, 1996). Here we report on the escape swimming and tailflicking behavior of the three larval and first postlarval stages of the lobster, *H. americanus,* and of the posthatching stage of the Australian crayfish, *C. destructor,* which is similar in size to the larval stages of *H. americanus.* The investigation was designed particularly to permit observations on the behavior of larval and juvenile animals to be compared with the larger literature on adult behavior to identify differences likely to reflect the different evolutionary selection pressures.

Materials and Methods

Animals

Larvae of the American lobster, *Homarus americanus,* were obtained from the lobster rearing facility at the New England Aquarium, Boston, Massachusetts; held in fresh aerated seawater at 8°C at the Marine Biological Laboratory, Woods Hole, Massachusetts; and fed *Artemia* twice a day. Holding aquaria were cleaned every second day. Work on the Australian crayfish, *Cherax destructor,* was conducted at the Zoology Department, University of Melbourne, Australia. Adult animals were obtained from a local supplier, held in large aquaria maintained at 15°–17°C, and fed dry prawnfood pellets weekly. Gravid females were isolated in 5-l buckets and the first motile and independent stage, ADI (adult immature following the nomenclature of Sandeman and Sandeman [1991]), was collected for experimentation.

Experimental cells and water jet

The behavior of the animals was filmed in individual acrylic plastic cells 40 mm wide × 40 mm long × 20 mm deep for larval and stage IV *H. americanus*, and 60 mm wide × 60 mm long × 15 mm deep for ADI *C. destructor*. Transparent walls allowed the video camera to be mounted looking vertically down or horizontally across the cell. For latency and habituation experiments, the animals were stimulated with a water jet delivered through a Pasteur pipette drawn to a fine tip (Schmitz, 1992). The pipette was connected to an elevated water reservoir by a silicon tube with a solenoid valve in the line. The duration of the jet (40 ms) was adjusted by controlling the solenoid with an electronic stimulator. The possibility of visual stimulation was reduced by using a fine clear pipette. Animals did not respond to its slow approach or proximity in the absence of a water jet. The amplitude of the water jet was controlled by adjusting the height of the water reservoir above the test cell. Three heights were used, which we termed low (50-cm elevation), intermediate (100-cm elevation), and high (200-cm elevation). These three heights produced water jets with velocities of 0.78 ± 0.037 mm/ms, 1.11 ± 0.007 mm/ms, and 1.48 ± 0.038 mm/ms (mean ± one standard deviation) respectively. The velocity, shape, and other characteristics of the water jet were characterized and calibrated by placing rhodamine dye in the reservoir and filming 10 jets.

Video filming

Videotapes were recorded with a Sony Hi8 video camera and played back on a high-resolution video recorder with single frame advance (Panasonic AG 6730). The camera had a set frame speed of 50/s (*i.e.*, a 20-ms sample rate) and an adjustable shutter speed that could range from 1/1000 s to 1/50 s (allowing each frame to be exposed for 1 to 20 ms). Slow shutter speeds (1/50 s) were used for determining the frame in which rapid movement first occurred because a blurred image was produced. Faster shutter speeds (1/1000 s) were used when sharp images were required or when it was important to determine how much movement had occurred between frames; for example, when viewing the dye front in water jet calibrations. White graph paper with 1-mm ruled squares was placed behind or beneath the cell during filming so that the animal's trajectory and speed of movement could be measured directly from the video image on a flat-screen monitor. Only tailflicks that took place in the plane perpendicular to the video camera were analyzed, and were easily identified. The behavioral recordings were played back frame by frame, and individual frames were selected for measurement, drawing, or other analyses. To measure the distance traveled by a tailflick, tracings of the animal's body were taken from the flat screen monitor relative to a selected point on the gridline background. The eye was chosen as a reference point on the animal for calculation of the linear distance traveled. However, the limitation of this method when calculating the distance traveled by LG tailflicks must be recognized, as the rotational component of this tailflick is not taken into account.

A light-emitting diode (LED) was placed in the field of view and connected to the delayed pulse output of the stimulator. By delaying the LED flash for a short interval after the signal that opened the solenoid valve, and placing rhodamine dye in the stream during calibration trials, we were able to synchronize the LED flash with the emergence of the clear stimulus stream from the end of the pipette during experiments. The only requirement for the accuracy of this method was that the distance between the tip of the pipette and the animal be constant at the moment the stimulus was triggered. This was standardized with the animal relative to the gridline background and checked on the video record; samples in which this condition was violated were discarded. Accuracy became less of a problem as we became experienced at manipulating the animals and the pipette.

Histology

Animals were fixed in Bouin's fixative, embedded in wax, sectioned at 7 μm, and stained with Mallory's triple stain for viewing on a light microscope equipped for microphotography.

Morphological measurements

Abdominal measurements were taken by pinning fixed animals out on an agar plate, and measuring dimensions through a graticule in a 10× eyepiece mounted on a dissecting microscope. Abdominal lengths were taken from the tip of the telson (including the setae for stages III and IV *H. americanus* and ADI *C. destructor*) to the anterior edge of the first abdominal segment.

Results

Time interval between stimulus and movement and form of the movements

Images of body position over time during tailflick responses by larvae and stage IV postlarvae of *Homarus americanus* showed that larval responses to our water jet stimulus differed in many ways from those of stage IV juveniles (Fig. 1). First, and most noticeably, the latency between the stimulus and the first tailflick movement was different. Latency data were compared by one-way ANOVA, followed by a Student-Newman-Keuls test for multiple contrasts. The earliest sign of movement in response to the water jet was never seen before 60 ms in the three larval stages, and was usually much later and very variable (mean 139 ± 116 ms) (Table 1). There was no

Figure 1. Comparisons of latency to response and initial escape movements by *Homarus americanus* at three stages of development: stage I larva, stage IV postlarva, and adult. The larval escape response is the same irrespective of stimulus direction and larval stage, so a representative stage I response is shown. Although stage IV animals are able to respond to anterior and posterior stimulation with medial giant fiber (MG) and lateral giant fiber (LG) tailflicks as shown here, most responses observed during this study were of nongiant fiber origin. None of the three larval stages displayed tailflicks that could be attributed to giant fiber activity. The blocked stimulus used on the stage I larva and the adult animals represents a mounted pin and a metal rod respectively. The clear stimulus used on the stage IV postlarva represents a water jet stimulus. Note the comparative speed with which stage IV animals are able to complete MG and LG flicks compared to the adult. This is due to the relatively shorter distance the giant fiber action potential travels in smaller animals.

difference in escape latency among the three larval stages ($P > 0.3$). Because of this absence of responses faster than 20 ms, we concluded that the three larval stages of *H. americanus* do not possess functional giant fiber tailflicks. In contrast, stage IV juveniles exhibited two classes of escape flip that started within 20 ms. The speed of these responses is comparable with the values for giant fiber tailflicks in the literature (see Wine and Krasne, 1972) and with our own brief measurements of adult *H. americanus* (data not shown). Furthermore, the MGF and LGF tailflick body movements were clearly distinguishable when the stimulus was applied to the anterior and posterior ends of the animal respectively (Fig. 1), and they were characteristic of the trajectories produced by MG and LG tailflicks (Wine, 1984). From these results we concluded that stage IV *H. americanus* has functional giant fiber tailflicks. Giant fiber flicks by stage IV *H. americanus* had tailflick latencies significantly faster ($P < 0.0001$) than those of the larval stages. Stage IV juveniles did not, however, usually respond to the water jet stimulus with a giant-fiber-mediated tailflick, and another class of responses with longer latencies indicated that they could also produce NonG tailflicks (Table 1). On average, these NonG tailflicks had a latency significantly longer than the mean latency of each of the larval stages ($P < 0.0001$).

Another striking difference between the responses of the larval stages and stage IV animals was that the first movement seen following the long response time was invariably an extension of the abdomen rather than a flexion (Fig. 1). We termed this movement a hyperextension because the abdomen was curved back dorsally, apparently as far as the segmental joints would allow. In all three larval stages, abdominal hyperextension prior to a tailflick was far greater than any degree of extension achieved by stage IV postlarvae. Furthermore, once maximum hyperextension had been reached, this position was held for an average of 127 ± 7 ms before flexion, and therefore displacement, began. If a larva made a second flick, that also was usually preceded by a hyperextension. If there were subsequent tailflicks, abdominal extension resembled that of postlarvae and did not involve a hyperextension. All three larval stages showed this behavior.

ADI *Cherax destructor* produced tailflicks in which movement was well under way in less than 20 ms. As in stage IV *H. americanus*, these tailflicks were concluded to be of giant fiber origin due to their short latency and characteristic trajectories. ADI *C. destructor* also produced

Table 1

Escape latencies to water jet stimulus by Homarus americanus *and* Cherax destructor

Species	Stage	Class of tailflick	*n*	Mean latency in ms (mean ± SD)
H. americanus	I	Nongiant	346	145 ± 101
H. americanus	II	Nongiant	282	135 ± 132
H. americanus	III	Nongiant	341	137 ± 115
H. americanus	IV	Giant	13	≤20
H. americanus	IV	Nongiant	278	195 ± 147
C. destructor	ADI	Giant	592	≤20
C. destructor	ADI	Nongiant	70	51 ± 14

Flicks that commenced within 20 ms were attributed to giant fiber activation, and an estimate of standard deviation of their latency was not possible due to the constraints of the camera. Flicks of a longer latency were attributed to nongiant fiber activation. *n* is the total number of responses observed for each class of tailflick by 6 animals of each species and stage.

NonG tailflicks with latencies of 60 ms or more. On average, ADI NonG tailflicks had latencies significantly shorter than *H. americanus* larvae and stage IV NonG tailflicks ($P < 0.001$). The latency and types of responses exhibited by ADI animals in response to water jet or mechanical stimuli were similar to those of adult animals (Fig. 2). Giant fiber tailflicks were also elicited far more regularly than in stage IV *H. americanus:* 89.4% of *C. destructor* tailflicks were due to giant fiber activation, but only 5.4% of *H. americanus* tailflicks were of a latency short enough to be attributed to giant fiber activity.

Trajectories and distances traveled following the different types of tailflicks

Unlike the MG and LG tailflicks of stage IV *H. americanus* and ADI *C. destructor,* the direction of an incoming stimulus or the location of first contact with the body did not appear to determine the trajectory of the path followed by the larvae of *H. americanus* during tailflick sequences; tailflicks appeared to be made in random directions. Statistical tests on the distance-traveled data were made by one-way ANOVA, followed by Student-Newman-Keuls tests for multiple comparisons. The first tailflick by stage I and II larvae moved them a very short distance (Table 2). Stage III larvae traveled significantly farther than stages I and II ($P < 0.05$), and MG-mediated tailflicks in stage IV animals were significantly greater than stage III tailflicks ($P = 0.001$). These MG flips were also directional, as described in the preceding section. LG flicks did not carry stage IV animals very far on the first flick because the rotational component pitches the animal upward and forward away from the posterior threat. The results for the first tailflick by ADI *C. destructor* were comparable with those of stage IV *H. americanus* (Table 2).

Table 2

Summary table of distance travelled on the first flick by Homarus americanus *stages I, II, III, and IV and* Cherax destructor *ADI*

Species	Stage	Class of flick	*n*	Distance travelled in ms (mean ± SD)
H. americanus	I	Nongiant	14	2.45 ± 1.00
H. americanus	II	Nongiant	17	2.60 ± 1.44
H. americanus	III	Nongiant	17	5.55 ± 1.77
H. americanus	IV	Medial giant	13	15.04 ± 3.64
H. americanus	IV	Lateral giant	3	6.37 ± 7.70
C. destructor	ADI	Medial giant	10	16.03 ± 2.32
C. destructor	ADI	Lateral giant	10	5.88 ± 2.14

n is the number of tailflicks measured.

We also examined the distance traveled during the first and second tailflicks in sequences of multiple tailflicks. All larval stages of *H. americanus* traveled farther on the second tailflick, whereas stage IV postlarvae traveled farther following the first (MG) tailflick (Fig. 3). Due to the rotational component of an LG flick, stage IV *H. americanus* and ADI *C. destructor* traveled farther on the second NonG flip. None of the recorded MG flips by *C. destructor* were followed by subsequent tailflicks (Fig. 3). On average, ADI *C. destructor* were able to travel 6.5 times farther than stage I *H. americanus* on the first MG flick (Table 2). Stage IV *H. americanus* MG and LG flicks did not differ significantly from ADI *C. destructor* MG or LG flicks respectively in terms of distance traveled.

Morphology of the tailfan and abdomen

We looked for reasons for the significant difference between the distance traveled by stage III larvae compared to stages I and II. The addition of setae and uropods at stage III (Fig. 4) would seem to allow stage III animals to generate more thrust during a tailflick, and the increase in abdominal size (and therefore muscle mass) would presumably allow these animals to generate the force required to drive the proportionally larger tailfan. Stage IV postlarvae have larger tailfans than stage III larvae (Fig. 4); the paddle-like

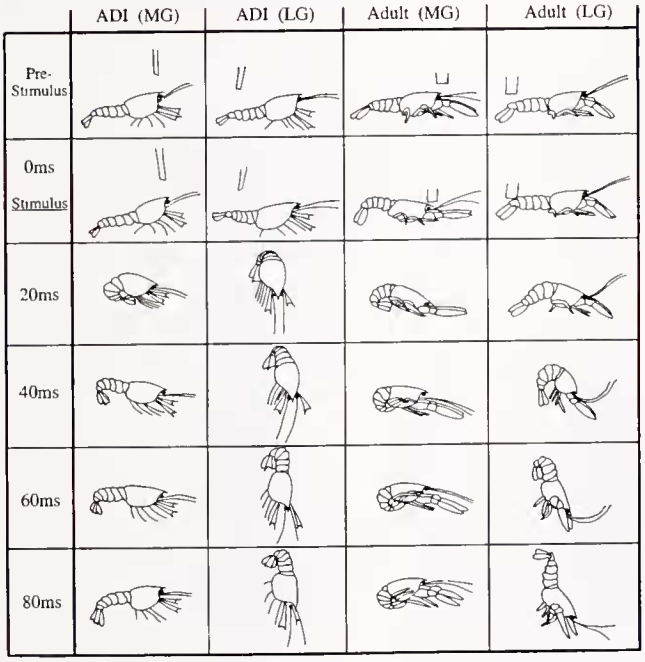

Figure 2. Comparisons of latency to response and initial escape movements by *Cherax destructor* at two stages of development: ADI and adult. ADI animals respond to anterior and posterior stimulation with essentially the same form of response shown to be due to medial giant fiber (MG) and lateral giant fiber (LG) activity in adult animals. Posterior and anterior stimulation were applied with a water jet to an ADI animal, and with a metal rod to an adult. Note the comparative speed with which ADI animals are able to complete MG and LG flicks compared to the adult. This is due to the shorter distance the giant fiber action potential travels in smaller animals.

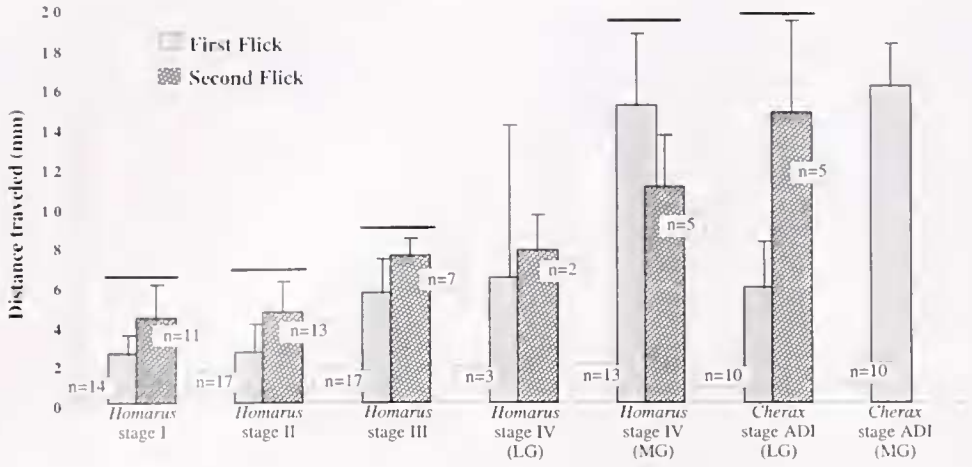

Figure 3. Distance traveled on the first two consecutive tailflicks following stimulation by *Homarus americanus* (stages I to IV) and *Cherax destructor* (ADI). Larvae and stage IV of *H. americanus* were stimulated with a mounted needle; *C. destructor* was stimulated with a water jet. Medial giant (MG) and Lateral giant (LG) flips were elicited in stage IV *H. americanus* and *C. destructor* by stimulating anteriorly and posteriorly respectively. Distances that differ significantly ($P < 0.05$) between the first and second flip are topped by a horizontal bar. Error bars are standard deviations of the mean. Data were tested for differences between the first and second flick within each stage by a two-tailed *t* test.

exopodites that are used by the larval stages to remain in the water column presumably increase drag during tailflicking.

A one-way ANOVA followed by a Student-Newman-Keuls test for multiple comparisons was used to test for differences between abdominal lengths; abdominal length is presumably a gross reflection of the muscle mass available for flexion. Interestingly, the abdominal length of stage I larvae of *H. americanus* does not differ significantly from that of ADI *C. destructor* ($P = 0.064$), although the latter are able to travel 6.5 times farther on the first tailflick (Fig. 3). In larvae of Stages II, III, and IV, the abdomens are significantly longer than in stage I ($P < 0.0001$; Table 3, Fig. 5).

Histology of the giant fibers

Transverse sections of the ventral nerve cord of the mid-abdomen of *H. americanus* larvae showed clear evi-

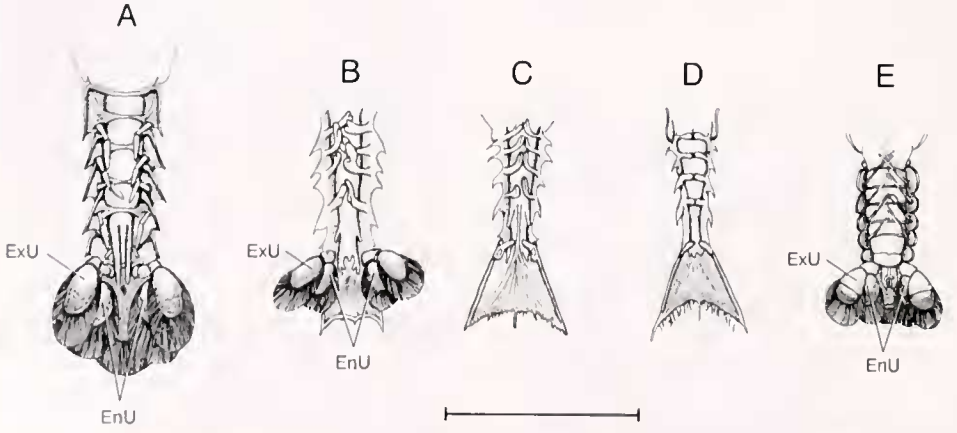

Figure 4. Ventral abdominal view of *Homarus americanus* stages IV, III, II, and I (A–D respectively) and *Cherax destructor* ADI (E). Note the increase in size that accompanies each successive molt in *H. americanus*, and that it is in stage III larvae that the endopodite (EnU) and exopodite (ExU) of the uropod first appear. These appendages make tailflicks more efficient and propel the animal a significantly greater distance than stage I or II larvae. Note the difference in size between *H. americanus* stage IV abdomens and *C. destructor* abdomens. *C. destructor* is able to travel the same distance as stage IV postlarvae, and over 6 times farther than stage I *H. americanus*. The setae of the pleopods in stage IV *H. americanus* and *C. destructor* have been omitted for clarity of presentation. Scale bar = 5 mm.

Table 3

Tailfan and abdominal dimensions for Homarus americanus *and* Cherax destructor

Species	Stage	*n*	Tailfan width in mm (mean ± SD)	Abdomen length in mm (mean ± SD)
H. americanus	I	13	2.24 ± 0.13	4.16 ± 0.28
H. americanus	II	13	2.36 ± 0.07	4.89 ± 0.21
H. americanus	III	5	2.48 ± 0.33	6.80 ± 0.80
H. americanus	IV	7	3.85 ± 0.35	6.81 ± 0.40
C. destructor	ADI	9	2.83 ± 0.19	4.36 ± 0.13

Tailfan widths for *H. americanus* stages III and IV and *C. destructor* ADI include uropods and setae, and abdominal lengths were taken from the tip of the telson (including the setae for stages III and IV *H. americanus* and ADI *C. destructor*) to the anterior edge of the first abdominal segment.

dence of the MG fiber in all three stages. No large-diameter axon was detected in the lateral region where the LG fiber is found in the adult (Fig. 6A); more than ten thousand sections were examined, from 6 stage I animals, 5 stage II animals, 6 stage III animals, and 3 stage IV animals. Unfortunately, the sections of stage IV *H. americanus* were not of a high enough quality to draw any conclusions. Both MG and LG fibers were clearly present in most of the sections taken from the same region in ADI *C. destructor* (Fig. 6B).

Habituation of tailflick responses

To investigate the habituation of the tailflick escape response in larval and postlarval *H. americanus* and juvenile *C. destructor*, we presented animals with a water jet stimulus at 1-min intervals and counted the number of tailflicks in response to each stimulus. Fricke (1984) also successfully employed a water jet stimulus to measure the rate of habituation in another species of crayfish, *Procambarus clarkii*. Although many other researchers use tactile stimuli to elicit tailflicks in crayfish (Krasne and Woodsmall, 1969; Wine and Krasne, 1969; Wine *et al.*, 1975), Wine and Krasne (1972) emphasize that the suddenness of onset of a stimulus is most important. We found that all animals continued to tailflick for 60 min in response to stimuli of the strengths used in these experiments. Because stimulus intensity can affect the rate of habituation, we examined the effect of water jets of three velocities. Groups of six replicate animals from each developmental stage were presented with one of three stimulus amplitudes at 1-min intervals for 20 min. Three animals from each group were stimulated a further 40 times. Animals were not reused in any experiments. Using repeated measures ANOVA over 20 stimuli, we found no difference between stages I to IV in the number of tailflicks executed over time (Table 4). However, there was a significant interaction between stimulus intensity and stimulus number, such that stronger stimuli initially evoked more tailflicks (Table 3 and Fig. 7). We fitted logarithmic curves to the data obtained for the three amplitudes of stimulation (Fig. 7) and found evidence for a slight initial habituation over time that eventually reaches a plateau so that animals continue to respond with multiple tailflicks even after 60 stimuli. Although stage IV animals did not differ from the three larval stages in the number of responses over time, the variability between the number of responses and stimulus number (as measured by the r^2 value) decreases with developmental stage (Fig. 8A–D). The trend towards habituation is also more obvious.

Unlike *H. americanus*, ADI *C. destructor* did not show

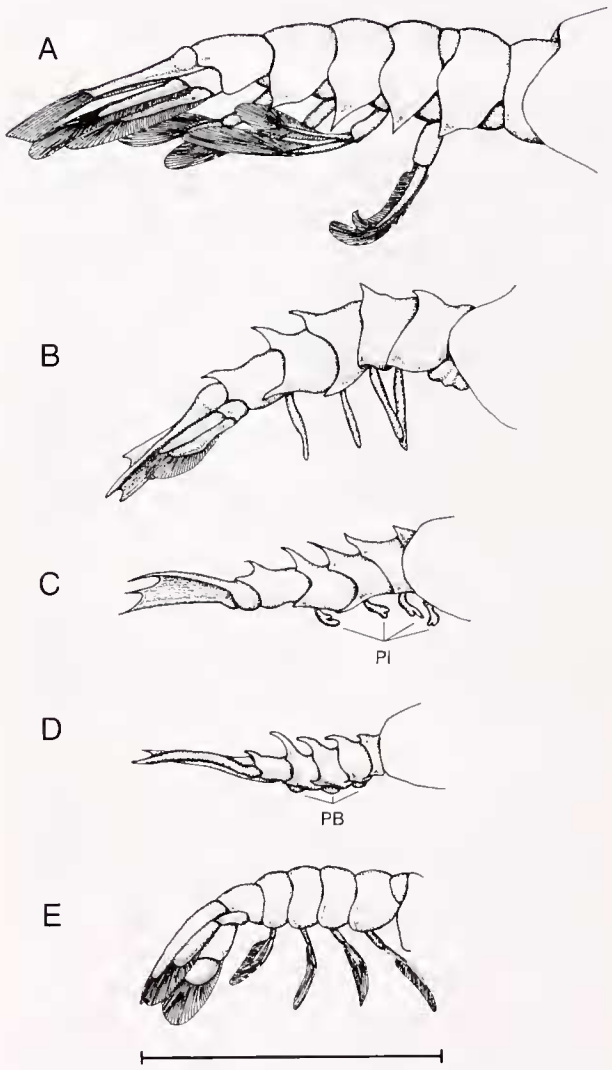

Figure 5. Lateral abdominal view of *Homarus americanus* stages IV, III, II, and I (A–D respectively) and *Cherax destructor* ADI (E). Note the increase in size that accompanies each successive molt in *H. americanus*. Pleopod buds (PB) are visible in stage I larvae and first appear as pleopods (PI) in stage II larvae, but are not used for swimming until stage IV. *C. destructor* hatches with functional pleopods and the full complement of adult appendages. Note the dorsal spines of the pleurites in the larval stages of *H. americanus* and that these spines are totally absent in stage IV animals and ADI *C. destructor*. Scale bar = 5 mm.

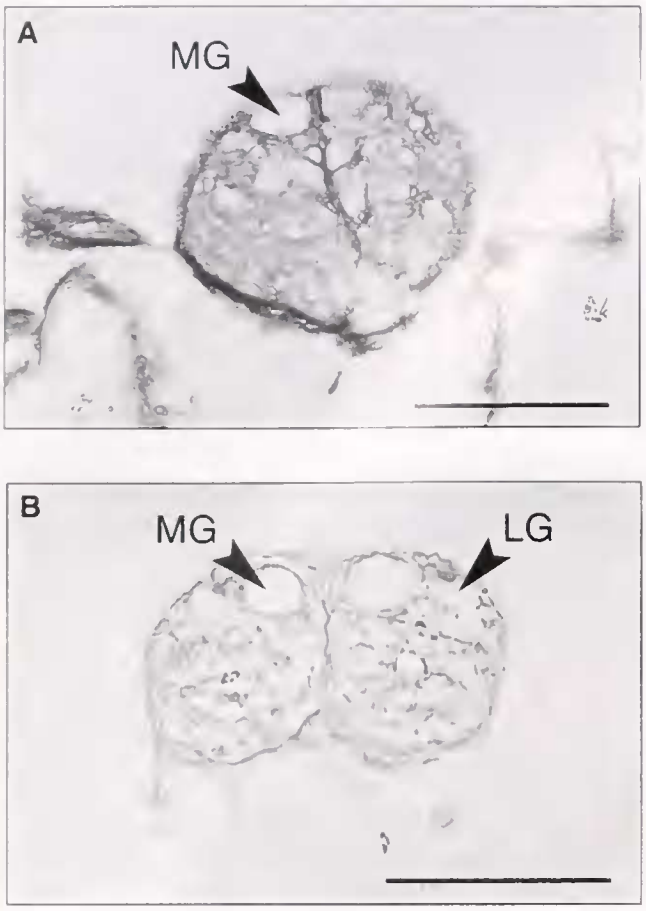

Figure 6. Sections (7 μm) of ventral nerve cords taken mid-abdomen, fixed in Bouin's fixative, and stained with Mallory's triple stain. Scale bar = 50 μm. (A) Sections taken from *Homarus americanus* larvae were the same irrespective of larval stage, so a representative stage II section is shown. Note the prominent medial giant (MG) fibers and the absence of lateral giant (LG) fibers. (B) Section of *Cherax destructor* ADI ventral nerve cord clearly showing the medial (MG) and lateral giant (LG) fibers.

any difference in the number of tailflicks over time to the three stimulus intensities used (Table 4B). ADI animals would typically respond with the same number of tailflicks on the 60th stimulus as on the first. This is reflected in the relatively low r^2 value (Fig. 8E).

Other observations

H. americanus larvae did not always respond to the water jet with tailflicks. Occasionally a different response was observed in which the abdomen was hyperextended (as in the hyperextension preceding a tailflick described above), the thorax was curled dorsally, and the chelipeds and pereiopods thrust laterally. This position was often maintained for as long as 1 s before a slow retraction of the abdomen, thorax, and lateral appendages. We termed this combination of hyperextension and lateral extension of the appendages "starburst" behavior. Starburst responses were not observed as often as tailflicks, but they occurred frequently enough to be of interest. Of the three possible responses (tailflick, starburst, or no response), starburst behavior occurred 13%, 21%, and 8% of the time for stage I, II, and III larvae respectively. Stage IV postlarvae and ADI *C. destructor* did not exhibit starburst behavior.

The LG tailflicks evoked by posterior stimulation of ADI *C. destructor* were often followed by a sequence of NonG tailflicks that carried the animal upward away from the bottom of the experimental tank. The animal would then sink slowly with its appendages extended. In larger holding aquaria, where objects such as PVC piping and rubble was present, the sinking trajectory appeared to be monitored by the animal to allow it to land on such objects. If the sinking trajectory needed adjustment to allow this to occur, the animal would initiate another tailflicking sequence (presumably NonG, although we have no way of knowing this) to bring it over the apparently desired landing site. When stimulated anteriorly, ADI animals produced MG flips that carried them in a flat trajectory backward and almost always occurred without further NonG flips.

Discussion

The tailflicking behavior of the larvae of *Homarus americanus* differs in a number of ways from that of juvenile and adult animals. Some of the differences revealed by our study are likely to reflect the different environments in which the animals live. One of the most interesting findings is that the larval stages do not exhibit giant fiber tailflick responses even though they have large medial giant fibers. This, coupled with the fact that the trajectory is not predicted by the location of the stimulus, suggests that tailflicking behavior in *H. americanus* larvae may advantage the animals in ways other than those normally associated with the behavior in adult animals. Our results suggest some possibilities. The hyperextension may be an important clue. The larvae remain in the water column and move slowly by beating the external rami, exopodites, of their biramous limbs (Ennis, 1995). The exopodites are paddle-like, with a fringe of setae (Herrick, 1909), and while this is an efficient arrangement for slow swimming (Laverack *et al.*, 1976; Macmillan *et al.*, 1976; Neil *et al.*, 1976), it is likely to

Table 4

Repeated measures ANOVA performed on Homarus americanus *and* Cherax destructor *habituation data*

Species	Interaction	Mean-square	*F*-ratio	*P* value
H. americanus	Stimulus × stage	40.39	1.53	0.217
H. americanus	Stimulus × intensity	120.38	4.57	0.015
C. destructor	Stimulus × intensity	0.70	1.155	0.333

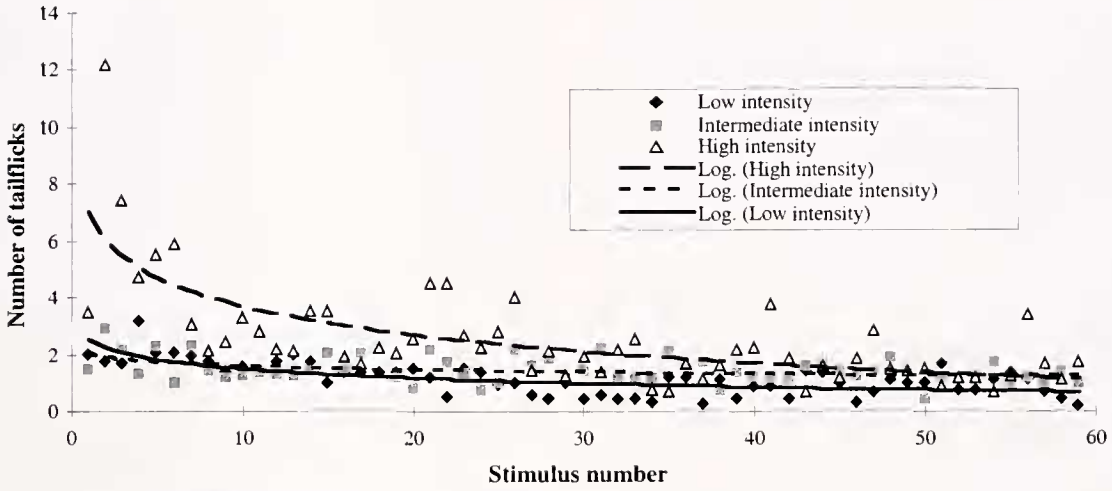

Figure 7. Mean number of tailflicks made by *Homarus americanus* in response to three stimulus intensities. The responses from stages I to IV were pooled for each stimulus intensity. A total of 21 animals (at least 5 from each stage) were stimulated at each intensity 20 times at 1-min intervals, and of these 11 (3 from stages I, II, and IV and 2 from stage III) were stimulated a further 40 times at 1-min intervals. A repeated measures ANOVA revealed that significantly more tailflicks are made in response to the high stimulus intensity for the first 20 min. Logarithmic curves were fitted by Excel 5.0.

increase viscous drag during tailflicking behavior. Drag is a significant issue in adult responses because animals reduce it by actively streamlining the legs during MG tailflicks (Cooke and Macmillan, 1985). The larval stages of *H. americanus* are unlikely to actively streamline the exopodites because the innervation and control of the limbs appears to be rudimentary (Hill and Govind, 1984; Macmillan, 1997). The hyperextension, a movement exhibited only by the larval stages, preceding the first tailflick may have evolved to overcome the drag created by the setae-laden exopodites, appendages specific to the larval stages; an initial tailflick of greater force may serve to passively streamline the exopodites and increase the displacement achieved by subsequent tailflicks. This hypothesis is supported by our finding that all of the larval stages travel significantly farther as a result of the second tailflick, whereas the first MG flick of stage IV animals, which lack exopodites, carries them significantly farther than the second flick. The comparison between ADI *C. destructor* and the larval stages also supports this hypothesis. ADI animals lack exopodites, have abdominal lengths similar to stage I larvae and significantly shorter than larval stages II and III, yet are able to travel farther (2.8–6.5 times) on the first tailflick. However, these comparisons of the tailflicking distance traveled by ADI *C. destructor,* stage IV *H. americanus,* and the larval stages must be made with the knowledge that these tailflick behaviors have different underlying neural mechanisms.

Because the hyperextended position is held for such a long time preceding flexion (127 ± 7 ms), therefore delaying displacement, the putative advantage it confers to streamlining the exopodites comes at a cost to a budget of total tailflicking time. Hydrodynamic factors are dominant features of the planktonic environment. Some feeding fish generate a bow wave that can carry material momentarily away from their bodies, the rate of water flow being dependent on the speed of approach (Lauder and Clark, 1984). Lobster larvae have been found in a number of fish and diving birds that would certainly produce a bow wave (Ennis, 1995). An initial sail-like hyperextension held by a larva for extended periods in response to an appropriate stimulus might increase the probability that the animal would be carried off in the bow wave of larger predators, thus conferring a selective advantage. Subsequent nondirectional NonG tailflicks could then help to keep the animal within any locally moving body of water, and would increase the handling time (in this case the time spent pursuing an individual larva) for visual predators.

In *H. americanus,* stage IV is considered to be the stage at which a benthic life is adopted. However, stage IV postlarvae are excellent swimmers and are found in the water column. The evidence concerning the proportion of time they spend in the water column as opposed to associated with the bottom is fragmentary (Ennis, 1995). One might have predicted that this transitional stage would exhibit some sort of transitional behavior; planktonic animals are faced with threats that can come from any direction in the surrounding sphere of water, whereas benthic ones deal largely with threats in the upper hemisphere, for which adult tailflicking is clearly appropriate. It is therefore interesting that stage IV has essentially adult escape behavior, albeit with different thresholds for activation (Lang *et al.,* 1977). ADI crayfish, in our holding tanks, spend more time on the bottom or on protruding objects than swimming. In this

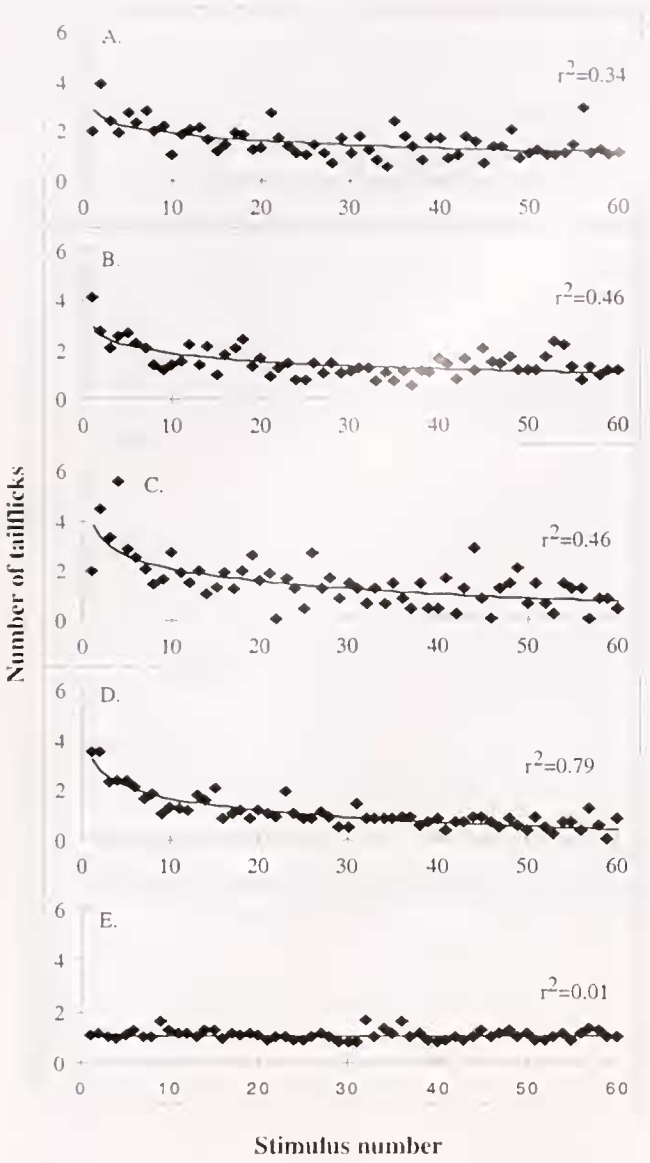

Figure 8. (A–D) Habituation of *Homarus americanus* stages I–IV to repeated stimulation at 1-min intervals. Three stimulus intensities were used and the results pooled for each developmental stage. Groups of six animals were stimulated 20 times at each stimulus intensity, and three animals from each group were stimulated a further 40 times. Although there is no significant difference between the four stages of *H. americanus*, the trend towards faster and more predictable habituation is clear through the increase in r^2 values with developmental stage. This trend suggests that the rate of habituation increases and becomes more predictable as animals grow. Fitted curves are logarithmic. (E) Habituation of *Cherax destructor* (ADI) to repeated stimulation at 1-min intervals. Three stimulus intensities were used and the results pooled. Groups of six animals were stimulated 20 times at each stimulus intensity, and three animals from each group were stimulated a further 40 times. The relatively low r^2 value indicates that there is no trend over time that differs significantly from the overall average number of tailflicks, *i.e.*, the mean number of tailflicks is as good a description of the data as the fitted logarithmic curve.

respect, our results suggest that both stage IV lobster postlarvae and ADI crayfish are more benthic than pelagic, but a more finely grained comparison with later stages might reveal subtle differences in these early developmental stages.

Our water jet stimulus did not always elicit a starburst response, but it did so with sufficient frequency that this response probably plays some part in the survival of the animals. Phillips and Olsen (1975) described a similar behavior in response to a touch stimulus by the pelagic puerulus larva of the Western rock lobster (*Panulirus longipes*) in which the animal "spreads its antennae to an angle of approximately 60° and the legs, abdomen and tailfan are also extended, while the animal remains motionless." Zoea larvae of the estuarine crab *Rhithropanopeus harrisii* also flare their antennal spines and flex their abdomens back over their carapace in response to a threatening stimulus (Morgan, 1987). Morgan showed, furthermore, that removal of the spines on zoea increases the probability that they will be eaten by small fish with a gape about the size of the larvae. We hypothesize that *H. americanus* larvae adopt the starburst posture as an antipredatory device, a theory supported by the observation that the numerous spines sculptured into the exoskeleton of the larval stages have been lost in stage IV animals, which do not exhibit the starburst behavior (Fig. 4).

These aspects of the tailflicking behavior of *H. americanus* larvae contrast sharply with those of stage IV lobsters and ADI crayfish. Both of these stages have essentially the same body form as their adult, although the proportions can be very different (Lang *et al.*, 1977) and, like the adult, they can execute a short-latency, directional response to threatening stimuli approaching anteriorly or posteriorly.

Our investigation into habituation is preliminary. From the extensive literature on habituation of tailflicking in crustaceans, we selected only a few aspects that one might expect to be different because of the differing ecology of the larvae and adults. Our data confirm earlier results (Lang *et al.*, 1977; Fricke, 1984, 1986) suggesting that habituation is an aspect of escape behavior in which one might expect to find subtle differences that have profound effects on the selective advantage to the animal. The larval and first postlarval stage of the lobster and the ADI of the crayfish will continue to tailflick to our threatening stimuli indefinitely. There are two possible explanations for this lack of habituation: there is no refuge in the plankton, and defensive behaviors are not likely to be effective against the types of predators that these animals face. The obvious conclusion from the results of the habituation experiment is that these animals cannot afford to habituate to this type of stimulus in the wild. Our results also predict that as later stages become equipped with larger chelipeds, the rate of habituation will gradually increase. This hypothesis is consistent with the work of Lang *et al.* (1977), who showed that smaller animals are more likely to employ escape behavior than larger animals, who are more likely to defend themselves.

The lack of GF tailflicks in the larval stages of *H. ameri-*

canus, despite the presence of large MG fibers, agrees with the work of Davis and Davis (1973), who recorded no giant fiber activity during rhythmic escape tailflicking induced by visual stimuli in semi-intact animals. Since the neuromusculature machinery for MG tailflicks is demonstrably present, a number of questions remain to be addressed. Are the MG fibers ever active in larval animals? At what stage do the LG fibers develop? What is the nature of the switch to GF tailflicks that occurs shortly after or during the molt from stage III larvae to stage IV postlarvae? These unresolved issues suggest that investigation of the development of the neural circuitry of this well-studied behavior is likely to be useful.

Our results provide a clear example of the way in which the different selective pressures operating on larval and adult forms within a species result in different behavioral outputs in response to similar sensory stimuli activating parallel neuromuscular systems. The differences between the species studied illustrate the way in which both the timing and the details of related behaviors can diverge markedly in related forms, again in response to a history of differing ecological and physiological conditions. We provide preliminary evidence for the neurobiological basis of some of the evolutionary changes, but this is an area that invites further investigation.

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